In The Name Of God

Investigation of Specific Targeting of Triptorelin Conjugated Dextran-Coated Magnetite Nanoparticles as a Targeted Probe in GnRH+ Cancer Cells in MRI

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Received: 20 January 2021

Accepted: 6 May 2021

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Journal metrics

Acceptance rate	58%
Submission to final decision	65 days
Acceptance to publication	40 days
CiteScore	3.200
Impact Factor	1.984

Abstract

the conjugation of superparamagnetic iron oxide nanoparticles (SPIONs), as tumor-imaging probes for magnetic resonance imaging (MRI), with tumor targeting peptides possesses promising advantages for specific delivery of MRI agents.

Purpose

Design a targeted contrast agent for MRI based on Fe3O4 nanoparticles conjugated triptorelin (SPION@triptorelin), which has a great affinity to the GnRH receptors.

1. Synthesized SPIONs-coated carboxymethyl dextran (SPION@CMD) conjugated triptorelin (SPION@CMD@triptorelin)
2. Characterized by DLS, TEM, XRD, FTIR, Zeta, and VSM techniques
3. Calculated the relaxivities of synthetized formulationsusing a 1.5 Tesla clinical magnetic field
4. Estimated quantitative cellular uptake, and cytotoxicity level of them

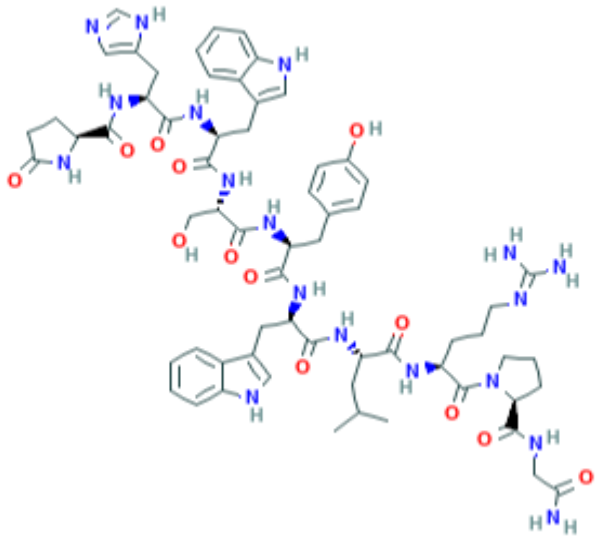
Introduction

- Cancers are one of the most frequent mortality causes worldwide due to the challenges arising in the diagnosis and clinical management.
- Conventional imaging techniques have played an effective role ☹ they suffer from low specificity.
- Targeted nanomolecular imaging has been proposed as a suitable solution for early detection.
- the detection of cancer based on specific biomarkers or receptors has resulted in substantial improvements in early and specific diagnosis.

- Several methods are used for the diagnosis of various human cancers.
- Imaging tests as a noninvasive way allow examining bones and internal organs.
- the most common imaging methods that are used in diagnosing cancer may include a computerized tomography (CT) scan, bone scan, ultrasound, X-ray, scan, and magnetic resonance imaging (MRI).
- The superparamagnetic iron oxide nanoparticles (SPIONs) are one of the US Food and Drug Administration- (FDA-) approved nanoparticles that are successfully used as tumor imaging probes for MRI.
- Used for drug delivery due to their low toxicity, biocompatibility, and demonstrating a great potential for theranostic.

- Coating of SPIONs with organic materials can also improve the colloidal stability.
- Conjugation of SPIONs with tumor targeting represents a promising platform for selective diagnosis of cancer biomarker/receptors and specific delivery of MRI agents.
- The specific binding of a peptide to its receptors (specific ligand-receptor interaction) which are overexpressed in cancer cells, resulting in efficient internalization of SPIONs based on receptor-mediated endocytosis (RME).
- Triptorelin is a synthetic decapeptide gonadotropin-releasing hormone (GnRH) agonis with similar structure to native GnRH and a great affinity to the GnRH receptors.

- Triptorelin is a potent inhibitor of the synthesis of testosterone (in men), an estrogen (in women) and is utilized to treat advanced prostate cancer and breast cancer.



https://pubchem.ncbi.nlm.nih.gov/compound/Triptorelin#section=2D-Structure 2021/June/25

Triptorelin

Medication

Triptorelin, sold under the brand names Decapeptyl and Gonapeptyl among others, is a medication that acts as an agonist analog of Gonadotropin-releasing hormone, thus reversibly repressing expression of luteinizing hormone and follicle-stimulating hormone. Wikipedia

Molar mass: 1,311.5 g/mol

Drug class: GnRH analogue; GnRH agonist; Antigonadotropin

ATC code: L02AE04 (WHO) QH01CA97 (WHO)

Excretion: Kidney

Formula: $C_{64}H_{82}N_{18}O_{13}$

https://www.google.com/search?q=triptorelin
2021/June/25

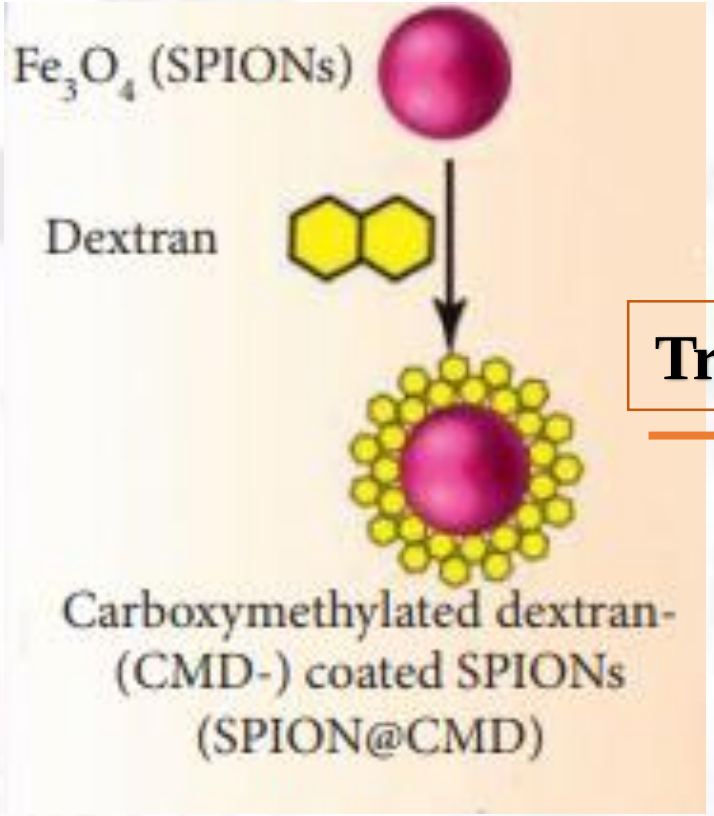
- The conjugation of dextran-coated SPIONs with triptorelin, as a targeting molecule, proper MRI probe for the tumor diagnosis.
- The triptorelin peptide was conjugated to synthesized SPION@CMD.
- the morphological properties and size dispersity of the prepared nanoparticles were assessed.
- The cytotoxicity of formulations was also investigated.
- In order to investigate the potential of synthesized SPION@CMD@triptorelin as a diagnostic nanoprobe, the MRI technique was carried out in vitro on MDA-MB-231 as GnRH-positive breast cancer cell line.

Materials & Methods & Results

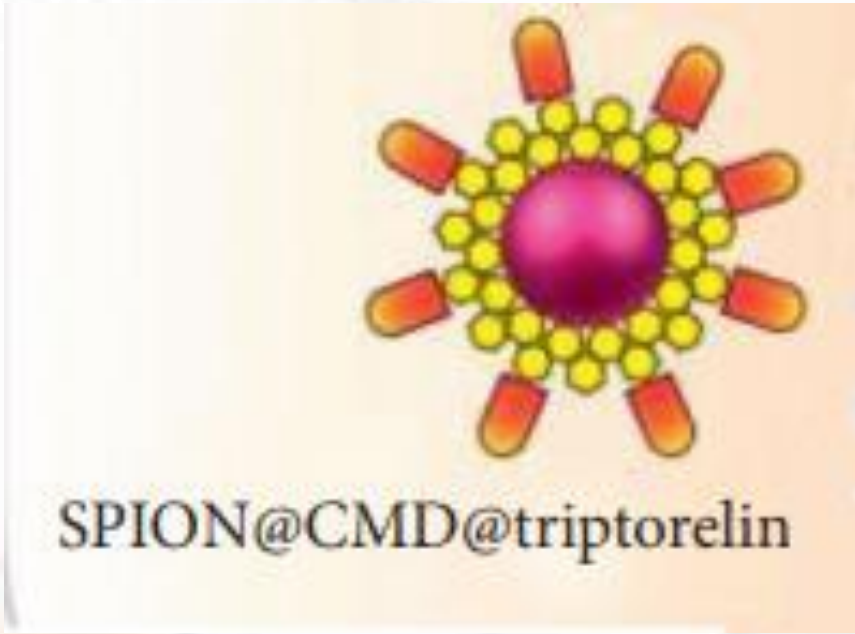
- All materials that were used for synthesis on nanoparticles and other analysis were obtained from Sigma-Aldrich Chemicals, USA.
- Cell culture media (RPMI1640), Fetal Bovine Sera (FBS), Trypsin, and penicillin/streptomycin solution were obtained from Gibco (Darmstadt, Germany)
- MDA-MB-231 cell line was obtained from the National Cell Bank of Iran, Pasteur Institute of Iran.

Step 1

Nanoparticle synthesis

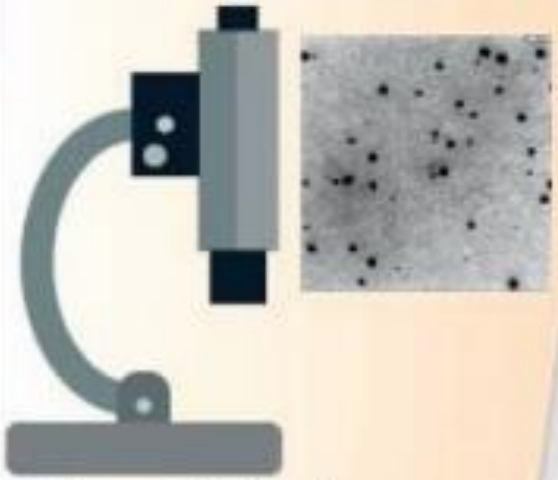


Triptorelin



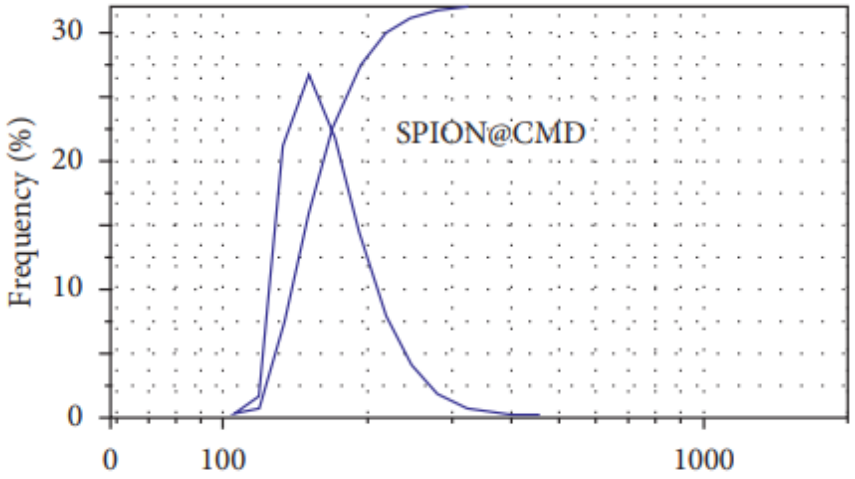
Step 2

Dynamic light scattering (DLS)

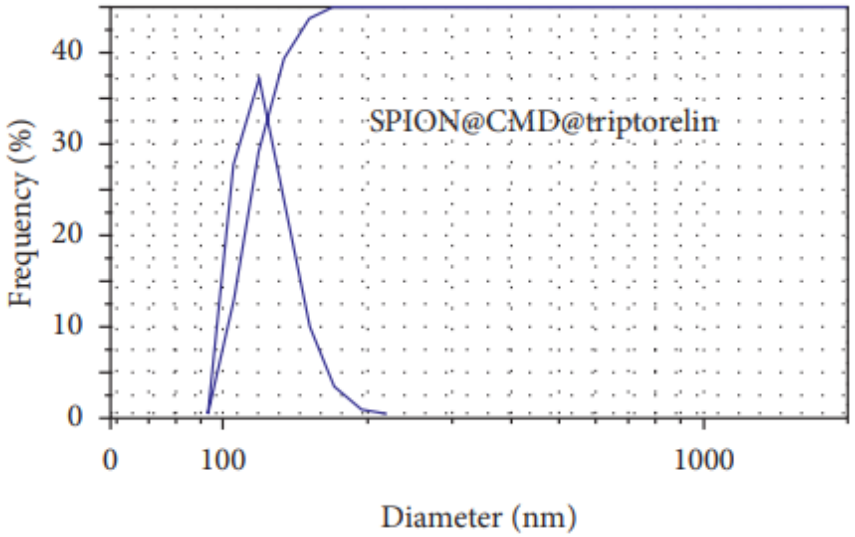


Transmission electron microscopy (TEM)

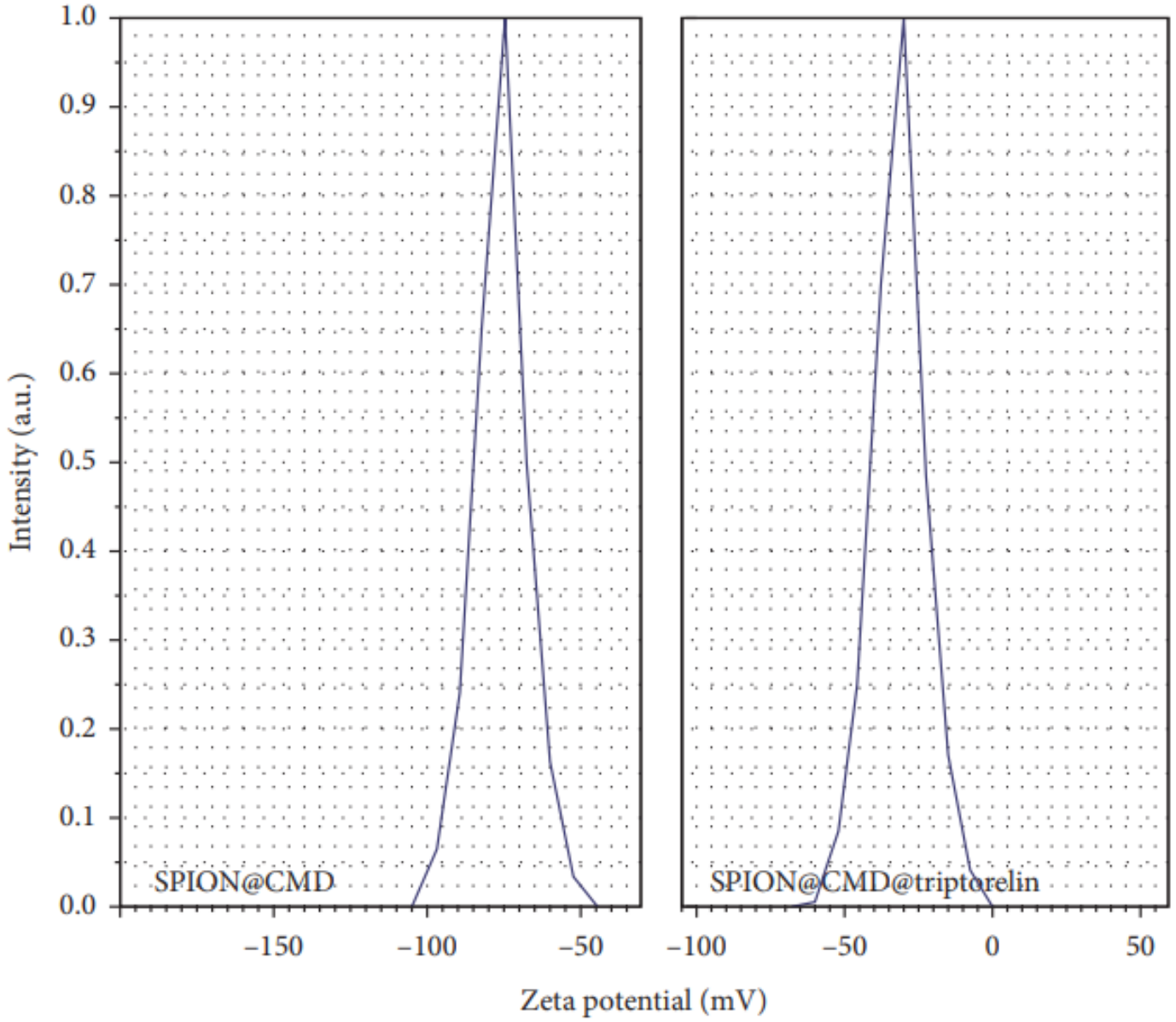
DLS Result



(a)



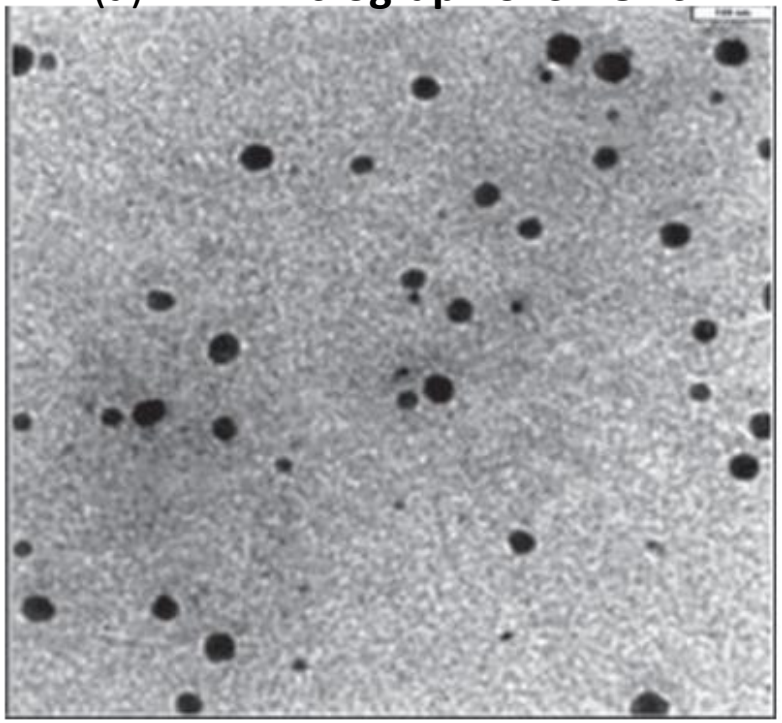
only one peak was observed in both SPION@CMD and SPION@CMD@triptorelin. The highest number of SPION@CMD and SPION@CMD@triptorelin complexes had a diameter of *160 nm* and *116 nm*, respectively. indicates narrow particle size distribution and monomodal population of the particles due in part to the contribution of triptorelin with SPIONs.



Zeta potential was measured at ~−72.4 mV for SPION@CMD and ~−31.5 mV for SPION@CMD@triptorelin.

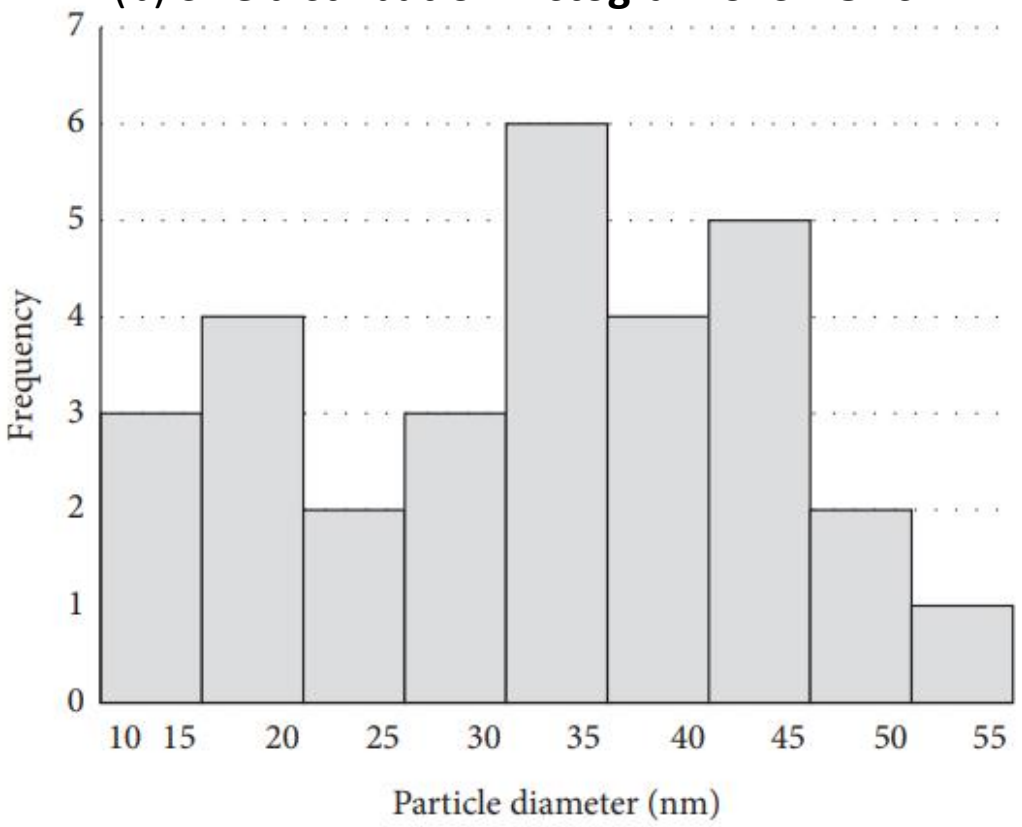
TEM Result

(a) TEM micrograph of SPIONs



(a)

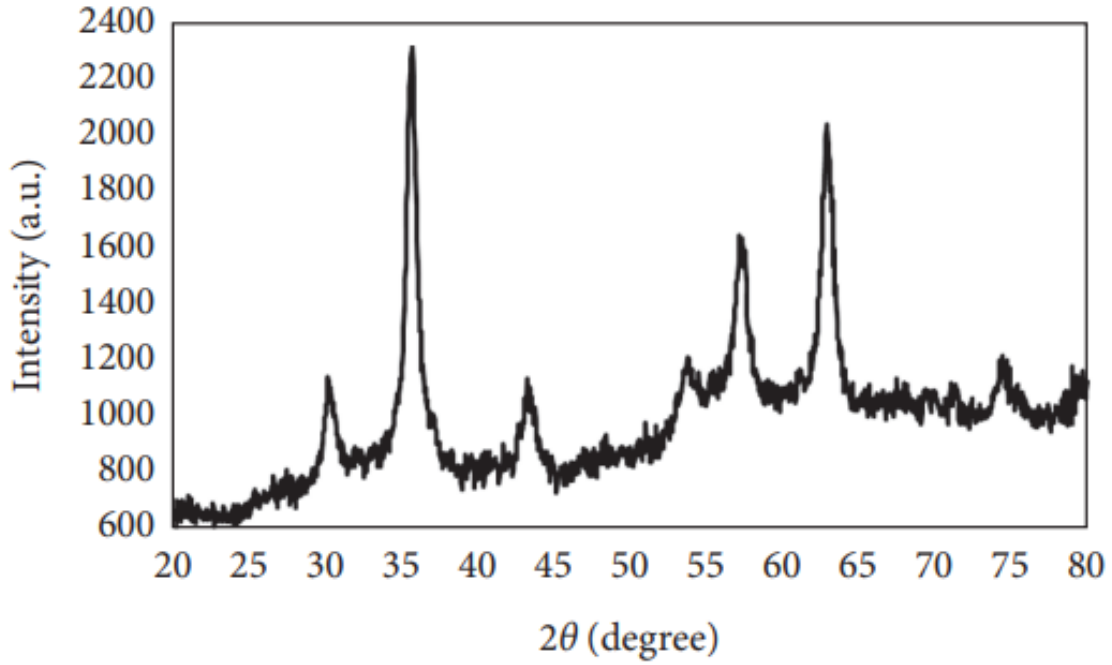
(b) Size distribution histogram of SPIONs



(b)

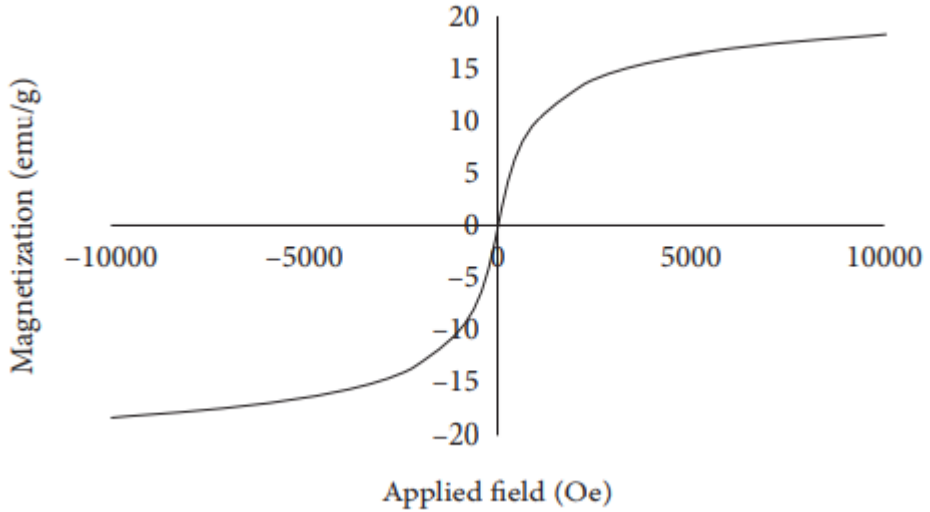
According to the TEM image, the synthesized SPION@CMD showed a spherical and homogeneous morphology. the particle size distribution histogram obtained from TEM image revealed that the largest number of SPION@CMD nanoparticles had dimensions between 30 and 45 nm with an average size of 31.35 ± 11.1 nm.

XRD Result



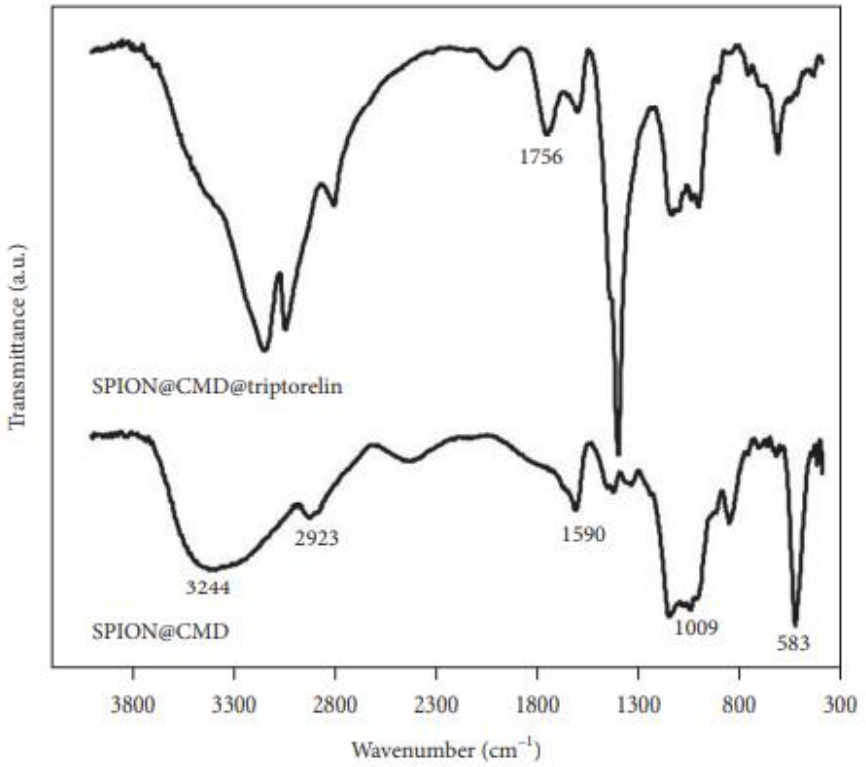
The XRD profile revealed that maximum XRD peak occurred at 2θ value of 35.7° that represented a typical SPION with an interlayer spacing value of 3.83242 A. The position and relative intensity of all peaks were matched with standard magnetite Fe3O4 pattern (JCPDS card, file no. 19–0629), indicating that the synthesized nanoparticles are magnetic (Fe3O4) crystals.

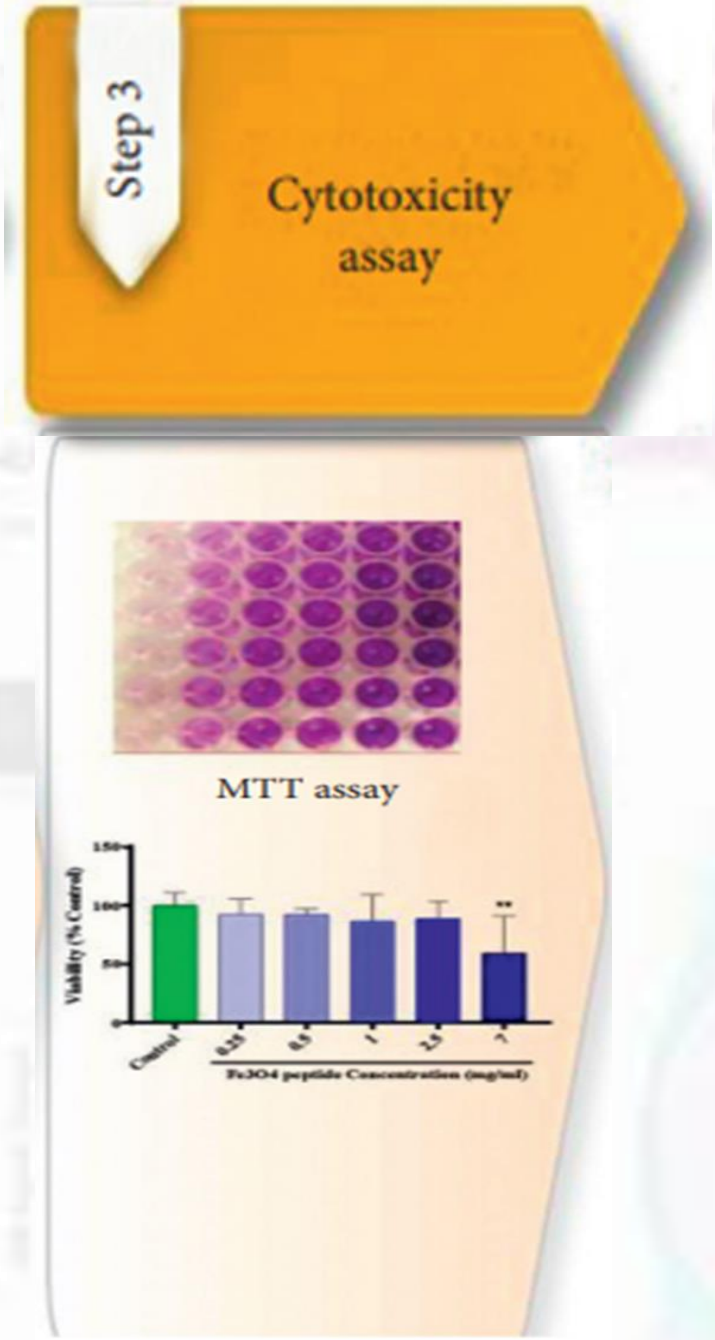
VSM Result



The **magnetic characteristics**.
that nanoparticles possessed superparamagnetism at 27°C with a saturation magnetization (Ms) value of 18.26 emu/g

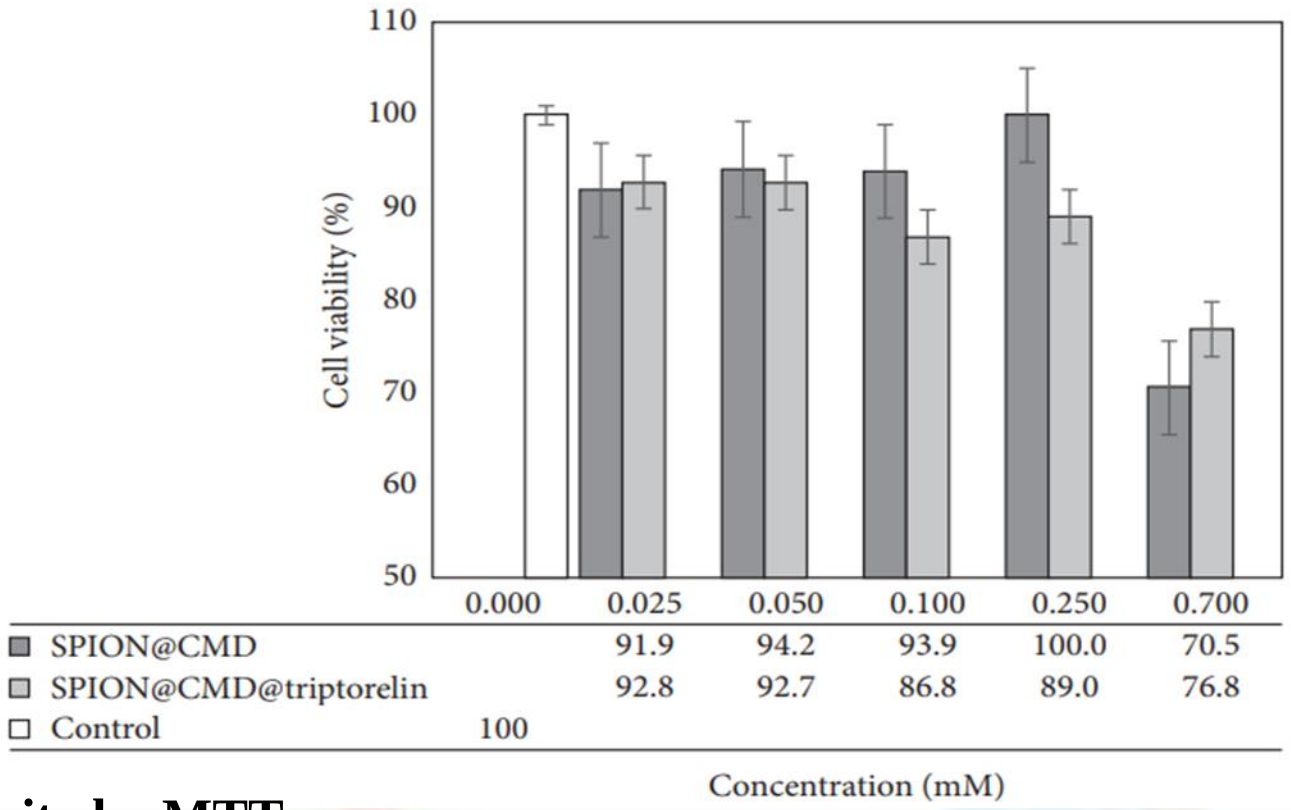
FTIR Result





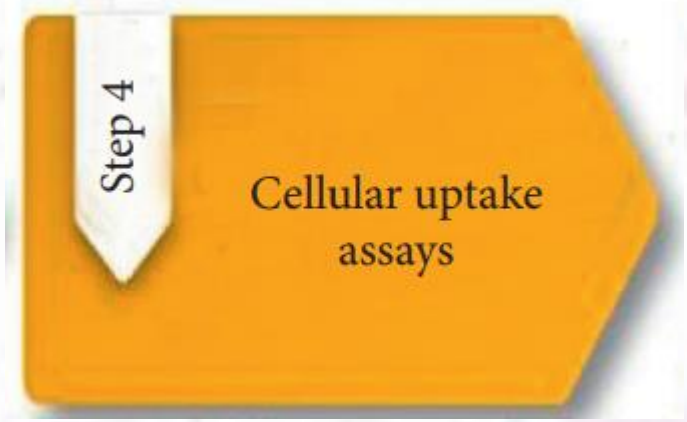
MDA-MB-231 cells were seeded at a density of 8 ×103 per well in 96-well plates and incubated overnight at 37°C with 5% CO2 in air. 0.025, 0.05, 0.1, 0.25, and 0.7 mM of SPION@ CMD and SPION@CMD@triptorelin were separately added to the culture medium and incubation was sustained for anadditional 24 h. The MTT assay was performed after 48 h. the absorbance was measured at a wavelength of 545 nm with a reference wavelength of 630 nm using an ELISA reader.

MTT Result



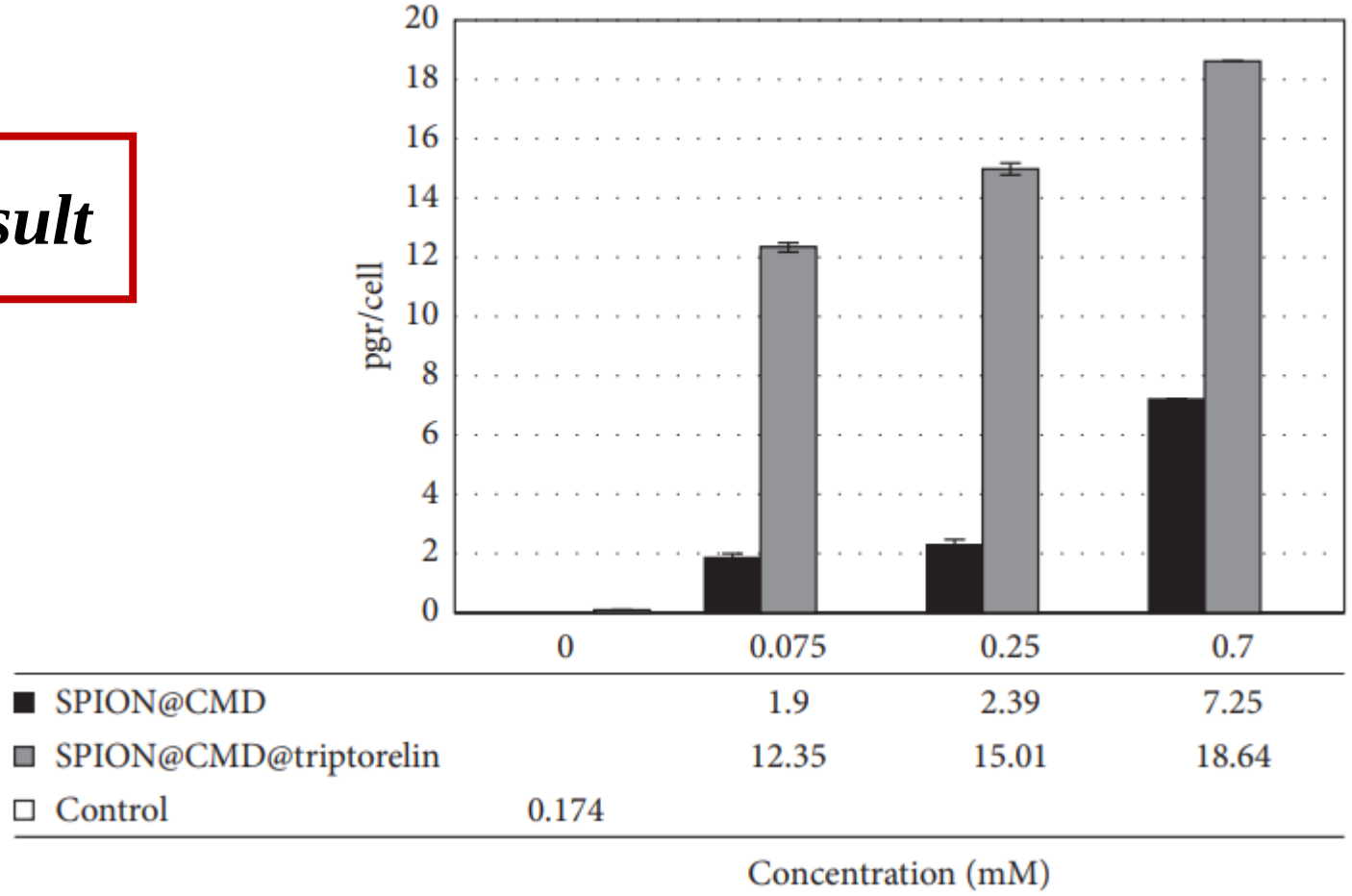
The **cytotoxicity by MTT** assay.

difference between cytotoxicity of SPION@CMD at concentrations of 0.025, 0.05, 0.1, 0.25, and 0.7 mM and control group was not significant ($*p > 0.05$), while 0.7 mM SPION@CMD showed a significant difference in comparison with the control group. The test also showed a survival rate of more than 60% for the maximum concentration of 0.7 mM SPION@CMD@triptorelin . confirmed the lack of cellular cytotoxicity of SPION@CMD@triptorelin formulation.



The MDA-MB-231 cancer cells were seeded in 6-well plates at the density of 4 ×105 cells/well and incubated overnight. Then incubated with 0.075, 0.25, and 0.7 mM of SPION@CMD and SPION@CMD@triptorelin for 24 h. washed three times with PBS and break down with perchloric acid. the concentration of Fe in cells was estimated with atomic absorption spectroscopy (AAS).

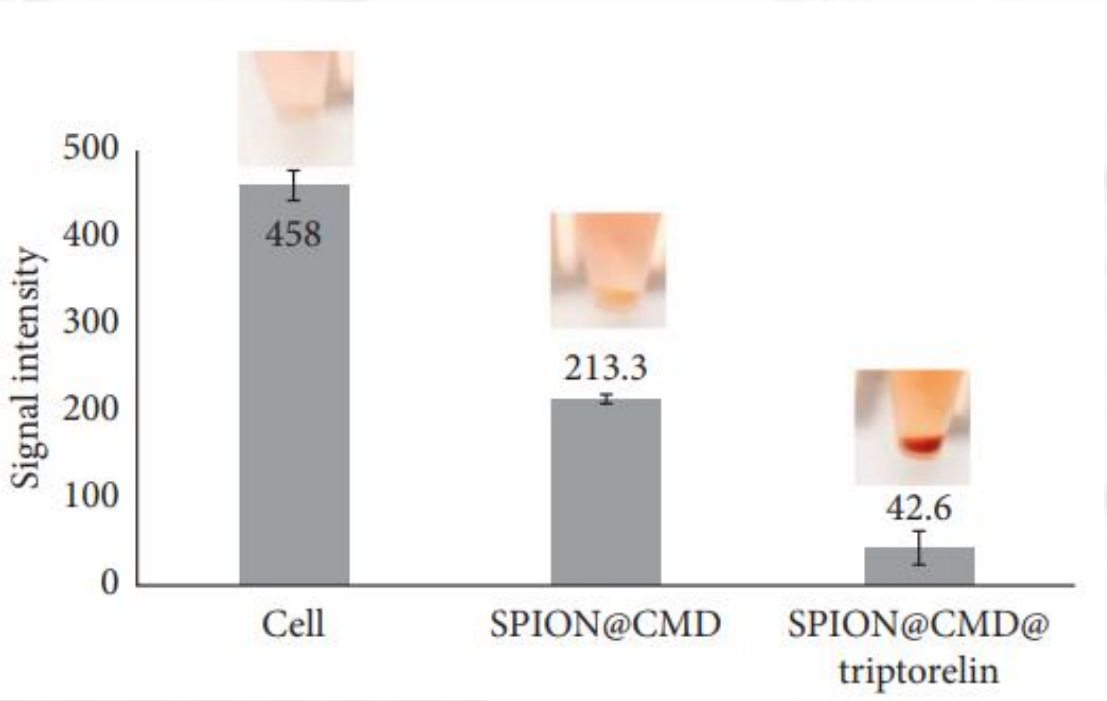
Result



According to the atomic absorption spectroscopy results, the intracellular iron of MDA-MB-231 cells treating with triptorelin-coated SPIONs was more than noncoated SPIONs . It is confirmed that the cellular uptake efficiency of targeted SPION@CMD@triptorelin was better than SPION@CMD

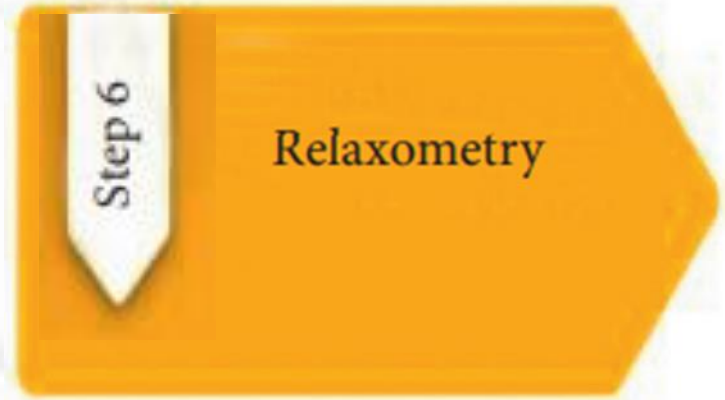
MRI technique was used to visualize the accumulation of prepared SPION@CMD and SPION@CMD@triptorelin in MDA-MB-231 cells. MDA-MB-231 cells (1.5 ×106) were seeded in T25 flasks and incubated overnight at 37°C. Following incubation, SPION@CMD and SPION@CMD@triptorelin with concentrations of 0.25 mM were added to each T25 flask and incubated for 24 h. the cells were washed with PBS, and 3 ×106 cells were placed in 2 mL tubes in 0.4% agarose solution. Cells in agarose without incubation with nanoparticles were used as the control. The T2-weighted images were obtained using a 1.5 T MRI scanner.

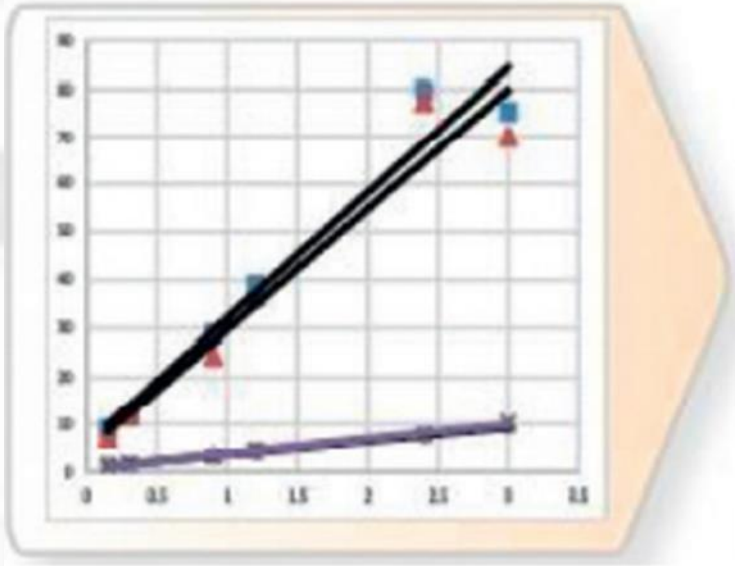
SPSS was used for data analysis.



Signal intensity depended on the nanostructures (SPION@CMD and SPION@CMD@peptide).
T2-weighted MR image in cellular medium with 24-hour incubation time

The ability of the synthesized nanoparticles to targeting specifically to the MDA-MB-231 cells was also confirmed with MR imaging techniques. The results demonstrated that nanoparticles functionalized with triptorelin reduced by more than 90% MR image intensity compared with the 53% reduction in SPION@CMD at a Fe concentration of 0.25 mM.





2.7. Relaxometry. The MR capability of SPION@CMD and SPION@CMD@triptorelin was carried out based on the relaxation rates ($\mathbf{R} = (1/\mathbf{T}_{1,2})$) which increase linearly with the SPIONs concentration according to the following equation:

$$\frac{1}{T_{1,2}} = \frac{1}{T_0} + r_{1,2}C,$$

Concentration of SPIONs

The relaxation rate of pure water

Longitudinal and transversal relaxivities' values (r1 and r2) were measured at 1.5 Tesla MRI scanner, using a phantom containing SPION@CMD@triptorelin with various concentrations of 0.15, 0.30, 0.9, 1.20, 2.40, and 3.0 mM.

T1 and T2-weighted images were obtained.

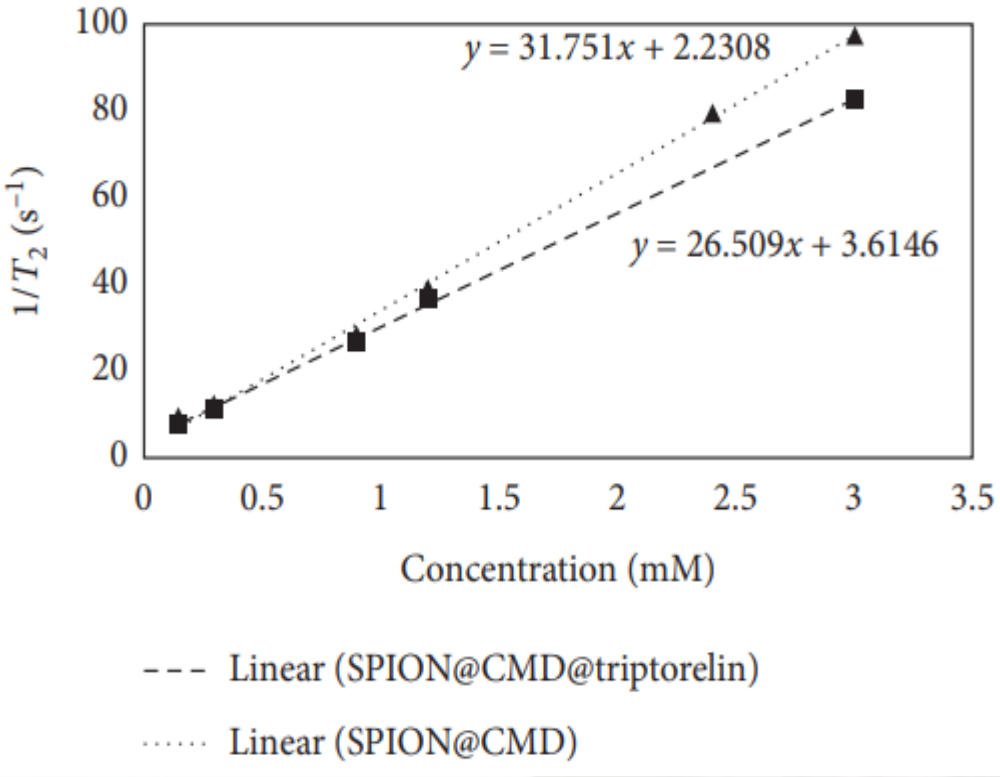
All curve fitting routines, which were used to determine the relaxation rate maps, were performed by Origin, Excel, and RadiAnt DICOM Viewer software.

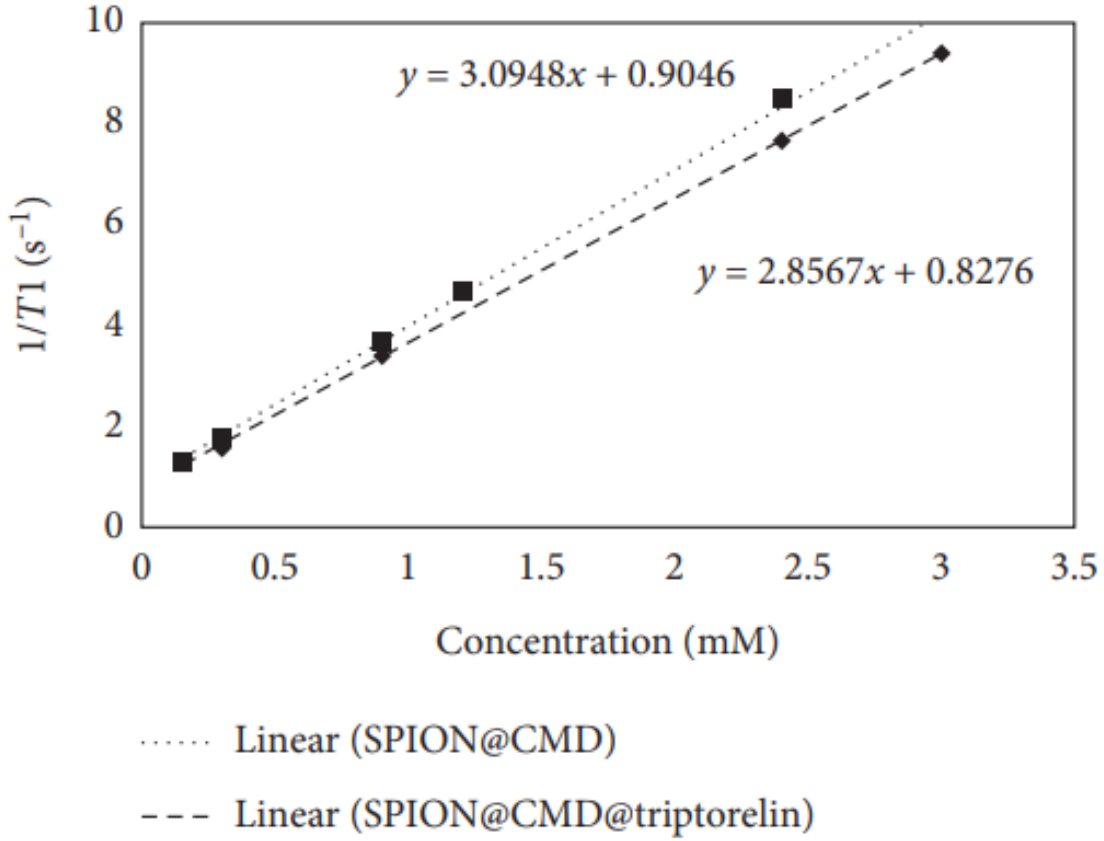
The software has the capability to open and display studies obtained from different imaging modalities:

- Digital Radiography (CR, DX)
- Mammography (MG)
- Computed Tomography (CT)
- Magnetic Resonance (MR)
- Positron Emission Tomography PET-CT (PT)
- Ultrasonography (US)
- Digital Angiography (XA)
- Gamma Camera, Nuclear Medicine (NM)
- Secondary Pictures and Scanned Images (SC)
- Structured Reports (SR)

Result

The r_2 relativity was calculated as $31.75\,mM^{-1}s^{-1}$ according to the linear plot slope of the SPION@CMD concentration depending on the inverse T_2 with $R^2 = 0.9965$, while the r_2 relativity was obtained as $26.51\,mM^{-1}{\cdot}s^{-1}$ with $R^2 = 0.9991$ for SPION@CMD@triptorelin which was lower than SPION@CMD





Calculated T1 relaxation rate and relaxivity at various Fe concentrations of SPION@CMD and SPION@CMD@triptorelin. T1-weighted images were acquired with fix TE of 8.7 ms and TRs ranging between 100 and 2000 ms for T1 measurement at various concentrations (0.15–0.3 mM) that were prepared in the deionized water, and deionized water played a role as a control sample

Discussion

- Targeted imaging probes is very important to improve diagnostic methods due to stability and biocompatibility.
- the main drawbacks of using SPIONs : aggregation and instability at physiological pH. To overcome these limitations, various surface coatings have been used to modify their surface properties.
- modification of SPIONs with carboxyl (-COOH) groups provide appropriate sites can be conjugated with any drugs and/or natural compounds containing amine (-NH2) group or their combinations.

- Contrast agents improve the quality of the images, but the injectable dose of them is limited.
- The utilization of ligands such as peptides can specifically increase the cell uptake by targeted cells ⟹ in applying lower concentration of the contrast agent.
- Recent studies have found that bare Fe_3O_4 nanoparticles could induce the cytotoxicity and apoptosis by ROS generation and oxidative stress. (by MTT)

- The evaluation of cellular uptake of formulations on MDA-MB-231 cell line indicated the importance of triptorelin peptide on the uptake of the targeted formulation.
- all concentrations, the cellular uptake for SPION@CMD@ triptorelin was SPION@CMD. (☺delivery of MRI agents with peptides increased the cellular internalization of functionalized-SPIONs in vitro)

- The mechanism of triptorelin-based internalization gives rise to receptor-mediated endocytosis, resulting in efficient internalization of formulation into the GnRH-expressing cells.
- cellular uptake of 7.25 pg/cell at the concentration of 0.7 mM (39.2 μg/ml) for the same cell line and incubation time in last study.
- the obtained average hydrodynamic diameters for SPION@CMD and SPION@CMD@triptorelin by DLS was 160 and 116 nm.

- The TEM image showed the synthesized SPIONs had uniform and heterogeneous morphology with an average size of 31 nm, while the size distribution histogram obtained from TEM image revealed also that the SPIONs as low as 10–20 nm.
- The small sizes of SPION@CMD in TEM image in comparison to DLS might be the determination of the hydrodynamic. diameter of the CMD-coated SPIONs via DLS in aqueous solution incorporating surface-bound water layers in their measurement, while TEM estimates the actual core size of dried SPIONs. the larger size in TEM image might be emanated from higher aggregation tendency of the smaller SPIONs due to Van der Waals forces between the particles.

MR images (T_2-weighted imaging) of SPION@CMD and SPION@CMD@triptorelin in an aqueous environment evaluated a noticeable contrast by changing the concentrations of SPIONs. Therefore, SPION@CMD@triptorelin could be considered as a targeted negative contrast agent. The r_2 value of SPION@CMD and SPION@CMD@triptorelin was estimated at 31.75 $mM^{-1}\cdot s^{-1}$ and 26.50 $mM^{-1}\cdot s^{-1}$, respectively, by a magnetic relaxometry at a 1.5 T conventional MRI system. The size, mass magnetization (M_S), and the magnetic field strength are factors affect the r_2 value [38, 39]. In agreement with previous studies, the obtained r_2 indicated that T_2 relativity also depends on the concentration of SPIONs. It was also demonstrated that the large sizes of SPIONs and stronger magnetic field lead to the higher r_2/r_1. The high value of r_2/r_1 ratio indicated T_2 contrast agent [40, 41]. In the present study, the calculated r_2/r_1 indicated SPION@CMD@triptorelin as a good candidate for a negative contrast agent in clinical magnetic field strength.

Conclusion

In the present study

SPION@CMD@triptorelin, as a targeted probe in GnRH + cancer cells, synthesized by a coprecipitation method and size of 31 nm

T_2 relaxivities (r_2) were determined to be 31.75 $mM^{-1}\cdot s^{-1}$ for SPION@CMD and 26.50 $mM^{-1}\cdot s^{-1}$ for SPION@CMD@triptoreln

in vitro cell viability assays: SPION@CMD@triptorelin showed no cellular viability reduction for up to 0.7 mM

- The findings suggested that SPION@CMD@triptorelin can be used in the future as a targeted theranostic agent for improving diagnostic and therapeutic application.

- It can be concluded that SPION@CMD@triptorelin loaded with anticancer drug provides a theranostic platform for specific delivery of MRI agents and drugs

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